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Review

Imaging biochemistry: applications to breast cancer

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Abstract

The use of magnetic resonance spectroscopy (MRS) to investigate breast tumour biochemistry *in vivo* is reviewed. To this end, results obtained both from patients *in vivo* and from tumour extracts and model systems are discussed. An association has been observed between transformation and an increase in phosphomonoesters (PMEs) detected in the ^{31}P MRS spectrum, as well as an increase in choline-containing metabolites detected in the ^{1}H spectrum. A decrease in PME content after treatment is associated with response to treatment as assessed by tumour volume. Experiments in model systems aimed at understanding the underlying biochemical processes are presented, as well as data indicating the usefulness of MRS in monitoring the uptake and metabolism of some chemotherapeutic agents.

Keywords: choline, magnetic resonance spectroscopy (MRS), ^{31}P MRS, ^{1}H MRS, phosphomonoesters

Introduction

The purpose of this section is to review the potential applications of magnetic resonance spectroscopy (MRS) to non-invasive probing of the underlying biochemistry of cells comprising a breast tumour. MRS does not generate an image of the tumour directly, but the spectroscopic data can now be obtained from a well localised area. Thus the biochemical information obtained from MRS can be interpreted in relation to a defined anatomical location, and images of metabolite distributions can be generated. In using MRS, the first aim is to identify surrogate biochemical markers of cellular transformation, thus differentiating benign tumours from malignant or potentially identifying the different tumour types. Subsequently, prognostic and diagnostic information is sought from the spectrum of malignant tumours. In prognosis, by yielding biochemical information on the tumour composition (eg the presence of a hypoxic or a drug-resistant fraction), MRS could allow the selection of an appropriate treatment. In diagnosis, early detection of tumour response to treatment, or monitoring of drug uptake and metabolism, could enable rapid optimisation of treatment if the tumour failed to respond. Overall, treatment that is better adapted to the individual patient could result from a better understanding of the biochemical events occurring within the tumour.

So far, most patient and model system studies have focused on ^{31}P MRS. Within the ^{31}P spectrum, the three nucleoside triphosphate (NTP) peaks, composed primarily of ATP, together with the inorganic phosphate (P_i) and phosphocreatine peaks, are indicative of energetic status. The P_i peak can also serve as a measure of intracellular pH. The phosphomonoester (PME) signal, which can be resolved into phosphocholine (PC) and phosphoethanolamine (PE) at higher field strengths or by decoupling, together with the phosphodiester (PDE) peak, composed of glycerol phosphocholine (GPC) and glycerol phosphoethanolamine, are indicative of lipid metabolism.

5-FU = 5-fluorouracil; GPC = glycerol phosphocholine; MR = magnetic resonance; MRS = magnetic resonance spectroscopy; NTP = nucleoside triphosphate; PC = phosphocholine; PDE = phosphodiester; PE = phosphoethanolamine; P_i = inorganic phosphate; PME = phosphomonoester.

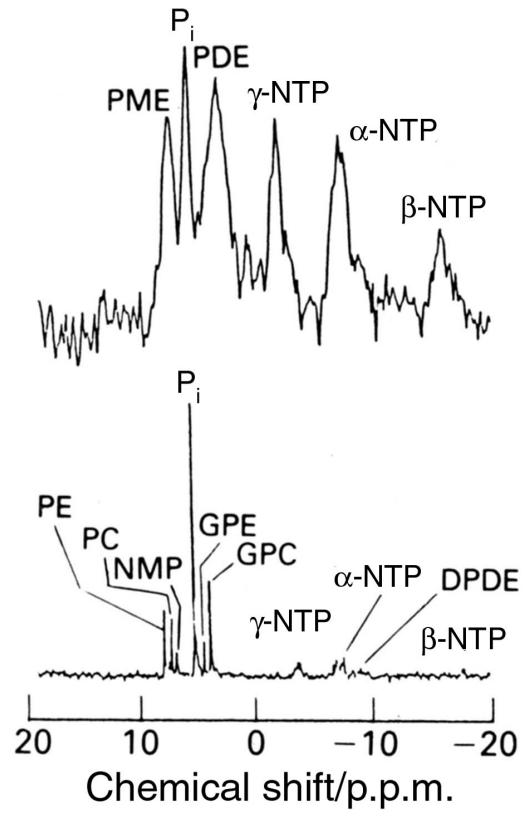
Finally, NAD(H) and uridine diphosphate sugars can also sometimes be resolved in the ^{31}P spectrum (Fig. 1).

In spite of its higher sensitivity compared to ^{31}P MRS, ^{1}H MRS has been used less frequently owing to the large signals from water and lipids that often dominate the spectrum, as well as the significant number of metabolite signals distributed over a relatively small chemical shift range. Nevertheless, when water-suppressed spectra are recorded, total choline, total creatine, lipids, glutamate, glutamine, inositols and lactate can be detected, potentially providing diverse biochemical information.

Other nuclei of interest include ^{19}F and ^{13}C. ^{19}F has been used primarily in studies of drug metabolism, particularly 5-fluorouracil (5-FU). The use of ^{13}C MRS in breast cancer has been restricted to model system investigations in which studies of the metabolism of specific pre-labelled compounds such as glucose or choline can be used to elucidate specific alterations in tumour biochemistry.

Considering spectra obtained from patients, these are often of poor sensitivity and resolution owing to technical challenges, the limited sensitivity of the method and low metabolite concentations. Consequently, the number of *in vivo* patient MRS studies is relatively limited, particularly in breast tissue. To identify the potential uses of MRS in the imaging of biochemistry *in vivo*, results obtained at higher field strengths from breast cancer tumour extracts will be discussed. Data obtained from model systems composed of homogenous cell populations or implanted tumour xenografts will also be presented, concentrating wherever possible on human breast cancer models.

Figure 1



^{31}P MRS spectra of human breast cancer tumour recorded *in vivo* at 1.5 T (top) and recorded *in vitro* after extraction at 5.8 T (bottom) [28]. DPDE, diphosphodiesters; GPE, glycerol phosphoethanolamine; NMP = nucleoside monophosphates. Reprinted from [28] by permission of the publisher, Churchill Livingstone.

Studies of tumour extracts, cells and xenograft models

Use of MRS to differentiate between benign and malignant tumours

In an effort to differentiate between benign and malignant tumours, early work concentrated on the ^{31}P MRS spectrum of human breast tumour extracts [1•,2]. These studies demonstrated higher levels of PMEs and NTPs in the carcinomas relative to benign tumours. Others have identified an association between the PC component of the PMEs and tumour grade as well as the proliferating fraction [3,4]. The importance of the PME region in identifying cellular transformation was further recognised by pattern recognition studies of spectra obtained from xenografts and compared with normal tissues such as liver, brain or muscle [5]. More recent studies comparing a normal human mammary epithelial cell line and a transformed human breast cancer cell line demonstrated that the levels of PMEs as well as PDEs were extremely low in the normal cells, and significantly less than in the breast cancer cell line [4]. A further assessment of PC as well as total choline content in a series of breast cancer cell lines demonstrated that levels of choline-containing metabolites are correlated with malignant transformation and progression [6] as well as the acquisition of a metastatic potential [7].

Such studies have recently been complemented by ^{1}H MRS investigations of extracts from non-involved breast tissue and breast tumours. The spectra demonstrated a high choline content as well as low levels of glucose and increased levels of lactate in the tumour spectra [8•]. Finally, spectra from about 200 fine-needle aspirate samples have shown that the choline peak in the ^{1}H spectrum could be used to distinguish between benign and malignant tumours with relatively high sensitivity and specificity [9].

Use of MRS in understanding tumour biochemistry

As recently reviewed [10•], it is still not entirely clear which are the metabolic changes that lead to the increased levels of PMEs observed in breast and other cancers. Changes have been hypothesised to be associated with an enhanced cell membrane synthesis, cellular

growth, or nutrient availability, as well as with cell signalling by lipid hydrolysis. Nevertheless, some understanding of the biochemical processes involved has been obtained by ^{13}C and ^{2}H MRS, in combination with labelled metabolites such as choline [4,11]. In breast cancer cells, rapid choline transport into the cells has been demonstrated, probably explaining the high levels of PC present in those cells. Furthermore, PC synthesis in quiescent breast cancer cells was slower than in proliferating cells, leading to lower PC levels in the non-proliferating population [4].

The rates of glucose uptake and lactate production have also been monitored with ^{13}C MRS and labelled glucose. Such studies demonstrate that the rate of glycolysis is affected by the proliferation state of the cell, as well as by hormone treatment, with glucose uptake reduced in quiescent cells and in cells treated with tamoxifen [4]. In other cell models, cellular de-differentiation [12] as well as apoptosis [13] were associated with changes in glucose uptake and metabolism that were detectable by MRS.

The chemical shift of the P_i peak enables the determination of pH within a sample. With the use of this information, MRS has been highly instrumental in demonstrating that whereas the extracellular pH of a tumour is acidic, its intracellular pH is in fact neutral to alkaline, leading to a reversed pH gradient in the transformed cell [14,15]. This finding affects the potential usefulness of some chemotherapeutic agents that are designed for optimum activity in an acidic intracellular environment, and some studies aimed at altering the pH gradient have been reported [16]. Another aspect of tumour physiology that can affect response to treatment, particularly radiotherapy, is oxygenation, and ^{19}F MRS of perfluorocarbons can be used in some cases to assess the presence of hypoxic regions within a tumour [15].

Use of MRS in monitoring response to therapy

The response to chemotherapy of experimental tumour models has been reviewed by Steen [17] and Daly and Cohen [18]. They show that untreated tumour growth was associated in several studies with an increase in the PME resonance and a decrease in the signal intensity of energy-rich metabolites. This trend is reversed after chemotherapy. After treatment and tumour shrinkage, a decrease in PME levels has been observed. Furthermore, an increase in NTP levels was reported after response to treatment and was termed by Steen tumour 'activation', a process potentially resulting from improved perfusion to the cells present in the residual shrinking tumour.

The use of MRS to detect the uptake and metabolism of the chemotherapeutic drugs themselves is also possible in some cases, as has been recently reviewed by Griffiths and Glickson [19]. 5-FU, which contains the magnetic resonance (MR)-visible ^{19}F nucleus, has been extensively investigated and with ^{19}F MRS it is possible to identify the catabolites of 5-FU in the liver, and to detect the parent drug as well as small peaks originating from the various nucleotides and nucleosides of 5-FU within the tumour itself. ^{19}F MRS has also been used to monitor the conversion of 5-fluorocytosine to 5-FU after transgene expression in a glioma model [20]. Others have been able to detect the expression of a virally transfected gene by monitoring the subsequent MR-visible build-up of a new metabolite in the ^{31}P MRS spectrum [21]. Finally, some studies of specifically labelled drugs such as ^{13}C-temozolamide have also been reported [19], demonstrating that this methodology could also be used to monitor drug pharmacokinetics *in vivo*.

Studies *in vivo*

Use of MRS in differentiating between benign and malignant tumours

Negnedank [22••] comprehensively reviewed the MRS studies *in vivo* performed on different types of human cancer. More recently, concentrating specifically on human breast cancer, Leach *et al* [23••] summarised the finding from nine different ^{31}P MRS studies *in vivo*. In line with the results obtained in model systems and described above, investigations *in vivo* demonstrate large PME and PDE signals in proliferating breast tumours. In 80% of breast cancers, PME signals, composed of both PC and PE, were higher than in normal breast, and PDE signals were higher in 77% of investigated tumours. If the P_i peak is taken as an indicator of pH, these reviews of the literature also demonstrate that breast tumours, like other cancers, show a slightly alkaline pH shift relative to control tissue. More recently, ^{1}H MRS studies performed *in vivo* have also demonstrated an increase in the choline metabolite peak, which reflects choline, PC and GPC levels. Roebuck *et al* [24••] and Kvistad *et al* [25] showed an increase in choline-containing metabolites in 70–80% of breast carcinomas, whereas only 14–18% of benign tumours demonstrated a detectable choline peak. However, choline was also detected in most breast-feeding volunteers.

Use of MRS in monitoring response to therapy

On the basis of the review by Leach *et al* [23••], response to therapy has been associated with a decrease in PME content in 14 of 17 patients, with all non-responding patients demonstrating an increase in PME levels. A further serial study by the same authors of 25 patients undergoing hormone, chemotherapy and radiotherapy treatments showed a significant correlation between a decrease in PME, PDE and total NTP levels and response to therapy as measured by a decrease in tumour volume [23••]. A multi-institutional trial is now in progress to confirm these results by extending localised ^{31}P MRS studies to investigate greater numbers of patients [26].

Considering the use of MR-visible chemotherapeutic drugs to monitor their uptake by the treated tumour, Wolf *et al* [27] recently published results obtained from different tumour types, including 26 cases of breast carcinoma. These studies demonstrated that after a bolus infusion of 5-FU, 'trapping' of the drug within the tumour region for relatively long periods (compared with drug in the blood pool) was strongly associated with tumour response to treatment, with 70% of trappers responding to treatment. None of the non-trappers demonstrated response to treatment.

Future perspectives

The results obtained from tumour extracts, cell models and models of implanted xenografts demonstrate the potential of MRS in assessing surrogate markers of transformation and response to therapy. However, clinical measurements so far have often been limited by the signal : noise ratio as well as the length of time required for measurements. Nevertheless, considerable progress has recently been made in this area. Improved automatic shimming and calibration methods lead to shorter examination times and therefore make spectroscopic studies more acceptable to the patient. The implementation of decoupling in the ^{31}P spectra has already led to an improvement in the separation of PE and PC signals *in vivo* as well as an improvement in signal : noise ratio by providing enhancement by the nuclear Overhauser effect. Recent measurements showing the practicality of ^{1}H spectroscopy in the breast indicate the potential to measure smaller tumours *in vivo* than has been possible with ^{31}P spectroscopy at 1.5 T. This also facilitates integrating such measurements into routine imaging studies because the same coil can be used. Most recently, new developments leading to clinical spectrometers with higher fields should improve both the sensitivity and the resolution of spectra *in vivo*. Together with improved localisation techniques, the ability to acquire signal from smaller voxels should lead to a better separation of tumour and normal tissue, and future studies might be able to generate enough metabolic information to provide both the diagnostic and prognostic parameters required by the clinician.

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